CLAIMS

1. MD-APCs which have the following properties :

- they present on their surface :

* antigen CD14 and CD64 with a mean intensity of about 5 to about 200,

* antigen CD80 and CD86 with a mean intensity of about 20 to about 200,

* antigen CD40 and mannose receptor with a mean intensity of 50 to 500,

- they are substantially devoid of the surface antigens CD1a and CD1c,

the presence and mean intensities respectively of CD14, CD64, CD80, CD86 being for instance determined by immunofluorescence staining and flow cytometry analysis,

- they present a phagocytosis property such as determined by the following test: said phagocytosis capacity being evaluated by an uptake of formalin fixed yeast, for example by culturing macrophages for 2 hours, adding yeast in 1/10 macrophages/yeast ratio and incubating at 37°C, 5% CO_2 atmosphere for 2-3 hours fixing by the May-Grünwald-Giemsa (MGG) staining, and the percentage of phagocytic MD-APCs being quantified for instance by microscopic analysis,

- they have the property of stimulating the proliferation of allogenic lymphocytes such as determined by the following test :

allogenic primary mixed lymphocytes reaction (MLR) was carried out in 96-well microtiter plates by adding different numbers ($2x10^3$ to $2x10^5$ in 100 μl medium/well) of MD-APCs to $2x10^5$ in 100 μl medium/well of allogenic T cells purified from buffy coats and after 5 days incubation at 37°C, cell proliferation was assessed by a colorimetric method, such as the hydrolysis of tetrazolium salt WST-1 (Boehringer Mannheim, Germany), (slightly red) to Formozan (dark red).

2. MD-APCs according to claim 1, which present, on their surface, antigen MHC-II with a mean intensity of about 100 to about 400, such as determined by immunofluorescence staining and flow cytometry analysis.

3. MD-APCs according to any one of claims 1 or 2, which are substantially devoid of surface antigen CD83, such as determined by immunofluorescence staining and flow cytometry analysis.

AMENDED SHEET

4. MD-APCs according to any one of claims 1 to 3, which present adherent properties such as determined by the following test :
the macrophages are cultured for 2 h in culture medium (I.M.D.M. or R.P.M.I.) on plastic flasks and the percentage (%) of adherent cells is quantified for instance by microscopic analysis.

5. MD-APCs culture wherein :
- about 10% to about 50% of the MD-APCs present antigen CD14 on their surface,
- about 10% to about 50% of the MD-APCs present antigen CD64 on their surface,
- about 30% to about 100% of the MD-APCs present antigens CD80 and CD86 on their surface,
- about 80% to about 100% of the MD-APCs present antigen MHC-II on their surface,
- about 70% to about 100% of the MD-APCs present adherent properties,
- about 30% to about 100% of the MD-APCs present a phagocytosis property,
each MD-APCs having the above-mentioned properties being such that said properties are expressed according to the intensities as specified in any one of claims 1 to 4.

6. Process for preparing a composition comprising MD-APCs according to any one of claims 1 to 5, comprising the culture of mononuclear cells in a culture medium containing chemical ligands of mononuclear cells, such as histamine or histamine agonist and a H_2 antagonist, in combination or not with "additional" GM-CSF, or other chemical ligands interacting with mononuclear cells and allowing differentiation into MD-APCs, such as detoxified LPS such as lipid A, C3 and other ligands of complement receptors, taxols, oxydoreductors such as flavenoids or polyphenols, ligands to CD40, to the TNF receptors or to vitamin D3 receptors.

7. Process according to claim 6, wherein the culture medium contains chemical ligands of mononuclear cells, for example histamine and cimetidine or a H_2 antagonist without "additional" GM-CSF, histamine being present at a concentration of about 10^{-2} M to about 10^{-6} M, preferably of about 10^{-4} M, and cimetidine or the H_2 antagonist being present at a concentration of about 10^{-4} M to about 10^{-9} M, preferably of about 10^{-6} M.

AMENDED SHEET

8. Process according to claim 6, wherein the culture medium contains chemicals ligands of mononuclear cells, for example histamine and cimetidine or a H_2 antagonist, in combination with "additional" GM-CSF, histamine being present at a concentration of about 10^{-2} M to about 10^{-6} M, preferably of about 10^{-4} M, cimetidine or the H_2 antagonist being present at a concentration of about 10^{-4} M to about 10^{-9} M, preferably of about 10^{-6} M, and additional GM-CSF being present at a concentration of about 50 U/ml to about 2000 U/ml, preferably of about 500 U/ml.

9. Process according to any one of claims 6 to 8, comprising :
- isolation of leukocytes, from healthy donors or from patients, from peripheral blood by apheresis and removal platelets and anticoagulant from the apheresis product,
- isolation of mononuclear cells (monocytes + lymphocytes) from red cells and granulocytes in order to have less than 10% granulocytes and less than 5% red cells,
- culture of the mononuclear cells obtained at the previous stage by placing them in an appropriate culture medium containing chemical ligands of mononuclear cells, such as histamine or an agonist of histamine, an H_2 antagonist, such as cimetidine, in combination or not with GM-CSF, for a time sufficient to obtain differentiated MD-APCs, preferably for about 5 to 15 days, and possibly separating the MD-APCs from the lymphocytes, and recovering the MD-APCs or the macrophages and lymphocytes.

10. Process according to any one of claims 6 to 9, wherein the culture medium of MD-APCs is added
- with crude antigens, for instance autologous tumor membrane, killed tumoral cells, bacterial capsides, viral homogenates cleared from nucleic acids,
- specific peptides against which an immune response is desired,
- cDNA or genetic material linked to vectors (for example gluconated polylysine) to allow transfection of the MD-APCs with material coding for the relevant peptide or protein to be presented on the macrophage membrane and against which an immune response is desired,
- or bispecific antibodies targeting on the one side, a surface antigen of the MD-APCs and, on the other side, a relevant antigen against which an immune response is desired.

AMENDED SHEET

11. MD-APCs liable to be obtained according to the process of any one of claims 6 to 10.

12. Pharmaceutical compositions containing as active substance, MD-APCs according to any one of claims 1 to 5 or 11.

13. Cellular vaccine compositions containing as active substance, MD-APCs according to any one of claims 1 to 5 or 11.

14. Medium containing elements necessary for the growth and differentiation of monocytes into MD-APCs according to claims 1 to 5 or 11, and in addition containing chemical ligands of mononuclear cells, for example histamine, cimetidine in combination or not with GM-CSF.

15. Cell processor or kit containing :
- means for the recovery of lymphocytes and monocytes free of contaminants,
- appropriate buffer and wash solutions and possibly appropriate means for the conservation of MD-APCs,
- means for preparing a culture for the monocytes and possibly the lymphocytes and containing chemical ligands of mononuclear cells, for example histamine, cimetidine or a H_2 antagonist in combination or not with GM-CSF,
- possibly means for transfection of cultured cells and means for targeting antigens to MD-APCs.

16. Cell processor or kit according to claim 15, containing :
- means for recovering and centrifuging blood to obtain a leukocyte concentrate,
- means for separating lymphocytes and monocytes from the other white cells and for eliminating the contaminating red cells,
- culture medium for MD-APCs and possibly lymphocytes with complements and particularly chemical ligands of mononuclear cells, for example histamine and cimetidine or a H_2 antagonist, in combination or not with GM-CSF,
- appropriate means for the conservation of MD-APCs,
- appropriate buffer and wash solution.

17. Products containing MD-APCs according to any one of claims 1 to 5 or 11, and lymphocytes, as a combined preparation for simultaneous, separate or sequential use in cell therapy.

AMENDED SHEET

18. Products according to claim 17, characterized in that they contain the MD-APCs and the lymphocytes in a ratio of at least 20% to 50% of MD-APCs expressed in cell number.

19. Method for the clinical treatment, comprising the administration of an appropriate amount of MD-APCs according to any one of claims 1 to 5 or 11, and preferably in an amount of about 10^8 to about $5x10^9$ MD-APCs.

20. Method according to claim 19 for the treatment of any disorder, comprising the administration of lymphocytes in an amount of about $4x10^9$ to about $10x10^9$ lymphocytes.

21. Use of chemical ligands of mononuclear cells, for example an agonist of histamine, in particular histamine, and a H_2 antagonist, in particular cimetidine, in combination or not with GM-CSF, for the preparation of MD-APCs having the following properties :

- they present on their surface :
  * antigen CD14 and CD64 with a mean intensity of about 5 to about 200,
  * antigen CD80 and CD86 with a mean intensity of about 20 to about 200,
  * antigen CD40 and mannose receptor with a mean intensity of 50 to 500.
- they are substantially devoid of the surface antigens CD1a and CD1c, the presence and mean intensities respectively of CD14, CD64, CD80, CD86 and the absence of CD1a and CD1c being for instance determined by immunofluorescence staining and flow cytometry analysis,
- they present high phagocytosis property such as determined by the following test : said phagocytosis capacity being evaluated by an uptake of formalin fixed yeast, for example by culturing MD-APCs for 2 hours to select adherent cells, adding yeast in 1/10 mMD-APCs/yeast ratio and incubating at 37°C, 5% CO_2 atmosphere for 2-3 hours fixing by the May-Grünwald-Giemsa (MGG) staining, and the percentage of phagocytic MD-APCs being quantified for instance by microscopic analysis,
- they have the property of stimulating the proliferation of allogenic lymphocytes such as determined by the following test :

allogenic primary mixed lymphocytes reaction (MLR) was carried out in 96-well microtiter plates by adding different numbers ($2x10^3$ to $2x10^5$ in 100 μl medium/well) of MD-APCs to $2x10^5$ in 100 μl medium/well of allogenic T cells purified from buffy coats and after 5 days incubation at 37°C, cell proliferation was assessed by a colorimetric method, such as the cleavage of tetrazolium salt WST-1 (slightly red) to Formozan (dark red) or such as Brdu incorporation during DNA synthesis.

Add G1 Add B1 Add C1 Add E5 Add H7